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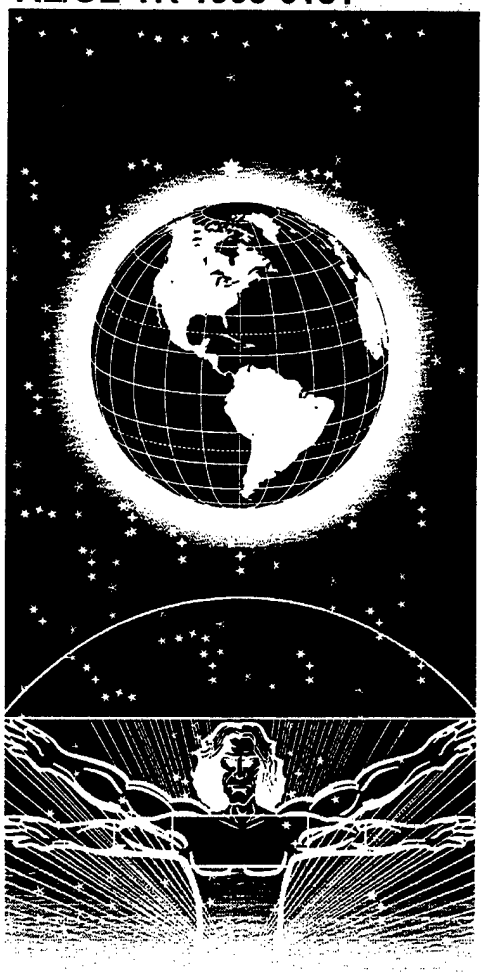

UNITED STATES AIR FORCE
ARMSTRONG LABORATORY

DOSE (AND TIME DEPENDENT) BLOCKADE OF PREGNANCY IN SPRAGUE-DAWLEY RATS ADMINISTERED AMMONIUM DINITRAMIDE IN DRINKING WATER

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November 1995

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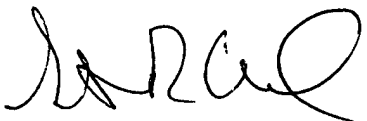
The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR



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Ammonium dinitramide (ADN) is a class 1.1 explosive oxidizer, which may be used in solid rocket propellant mixtures and explosives. A 90-day general toxicity/reproductive screen performed on this compound at doses of 162, 103, 29, and 0.0 mg ADN/kg/day resulted in a treatment-related adverse effect on litter production. Incidences of animals producing litters (1/11, 3/12, 12/12 and 11/12, respectively) and mean numbers of pups per litter (7, 7, 16 and 15, respectively) were both statistically significantly less than controls. In a follow-up study, mated dams treated with ADN at the same doses and examined at Gestation Days 10 and 20 showed a similar effect in fetus loss as those in the reproductive screen. A pre-versus postimplantation dosing regimen indicated that implantationis vulnerable to ADN effects during the preimplantation period (Gestation Days 1-3). No implantation sites were found in the rats treated with 2000 mg ADN/L drinking water during Gestation Days 1-3. Numbers of implantation sites found in the rats treated during Gestations Days 4-8 were similar to those found in the control group. The pituitary was not specifically identified in this study as the site of primary action, but serum progesterone was reduced by 27%, which may have resulted in reduced fertility.

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PREFACE

This is one of a series of technical reports describing results of experimental laboratory programs conducted at the Toxic Hazards Research Unit, ManTech Environmental Technology, Inc., located at Wright-Patterson Air Force Base, OH. This document serves as a final report on the dose and time dependent blockade of pregnancy in Sprague-Dawley rats administered ammonium dinitramide in drinking water. The research described in this report began in August 1994 and was completed in April 1995 under Department of Defense Contract No. F33615-90-C-0532 (Study No. F29A). Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division. This study was sponsored by the U.S. Army under the direction of CAPT Clay R. Miller, USAMRD/WRAIR, USA.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

The authors gratefully acknowledge Richard J. Godfrey, Jerry W. Nicholson, and Margaret Parish for their excellent technical assistance. Also acknowledged is Carlyle D. Flemming for statistical analysis of the data.

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ABBREVIATIONS

ADN	Ammonium dinitramide
BUN	Blood Urea Nitrogen
C	Celsius
dL	Deciliter
DOT	Department of Transportation
g	Gram
G	Gestation
GD	Gestation Day
h	Hour
IU	International Units
kg	Kilogram
L	Liter
mg	Milligram
mL	Milliliter
mmol	Millimole
N	Number
n	Nanogram
p	Probability
pg	Picogram
PM	Premating
RO	Reverse osmosis
SEM	Standard error of the mean
wt	Weight
vol	Volume

SECTION I

INTRODUCTION

Ammonium dinitramide (ADN) is being considered for use in solid rocket engine propellant mixtures and explosives. A minimal amount of acute toxicity data are available on ADN. Rabbits treated dermally at the EPA limit dose of 2 g/kg body weight survived a 14-day observation period. The oral LD_{50} of ADN in male Fischer 344 rats is 832 mg/kg (Kinkead and Wolfe, 1994a). Mortality occurred within an hour of gavage treatment and death was preceded by convulsions. Field reports on exposed personnel indicate that the compound is readily absorbed by the skin, resulting in numbness of the fingers (Koppes, 1993).

A general toxicity and reproductive screen has been performed in which male and female Sprague-Dawley rats received ADN in drinking water, both before and during pregnancy (Kinkead et al., 1995). Treatment-related hemolytic anemia was noted in female rats; treatment-related hypokalemia was noted in both sexes. The major effect noted in the study was a dose-dependent blockade of pregnancy. Only 9 and 25% of female rats treated with 162 or 103 mg ADN/kg/day, respectively, produced litters. Litter sizes were significantly smaller than those produced by the low-dose (29 mg ADN/kg/day) and control groups. Histopathologic examination of reproductive organs of ADN-treated rats revealed no lesions which would preclude production of live litters.

Continuous treatment with ADN has toxic effects on the female reproductive system. Although the reproductive organs appear to be unaffected, the majority of the rats treated at the high levels failed to produce litters. Because the dams gained weight in the normal pattern of gravid rats for the first seven days following mating, it appeared as though a decidual response occurred and that fetal death and/or resorption were occurring sometime after Gestation Day 7. To determine if the fetuses were being resorbed during early pregnancy, mated female rats were treated with ADN in a similar manner as the original study, then necropsied following 10 or 20 days of gestation. Fetuses, resorption sites, and corpora lutea were counted at necropsy.

In a follow-up study, to assess the effect of ADN on the preimplantation development of uterine receptivity and on postimplantation pregnancy maintenance, a pre- versus post-implantation protocol was performed following the method of Cummings (1990). Following mating, dams received ADN-treated drinking water daily, during days 1-3 or during 4-8 of pregnancy. Assessments were performed at necropsy on Gestation Day 9.

SECTION 2

MATERIALS AND METHODS

TEST COMPOUND

The ammonium dinitramide [$NH_4N(NO_2)_2$] was supplied by SRI International, Menlo Park, CA. Because ADN is a DOT explosive class "A" compound, only limited quantities were stored and no archive sample was maintained. The ADN sample was reported to be contaminated with 1 to 2% ammonium nitrate (Koppes, 1993). The test compound, a water-soluble powder, was maintained in an enclosed cabinet due to light sensitivity (Koppes, 1993).

PREPARATION OF DRINKING WATER SOLUTIONS

Solutions were prepared by weighing a specific quantity of ADN and adding it to a known volume of animal drinking water (wt/vol). The animal drinking water was supplied by a commercial water conditioning system (Osmotics Incorporated, Minnetonka, MN). This system consists of an activated carbon filter, softener, and reverse osmosis (RO) filtration. The pH of the RO water prior to the addition of ADN ranged from 6.5 to 7.0. Analyses of drinking water solutions are described in Kinkead et al., 1995.

STUDY I

Group Assignments and Dose Levels

Group	Number of Female Rats	Concentration of Administered ADN (mg/L Drinking Water)	Target Doses of ADN (mg/kg Body Wt/Day)[a]
Control	20	0	0
Low	20	200	16
Middle	20	1000	80
High	20	2000	160

[a]Assumed daily water consumption of 40 mL/500 g rat

Test Animals and Clinical Measurements

Forty male and 80 female Sprague-Dawley derived outbred albino rats [Crl:CD®BR] known as Charles River CD rats, were purchased from Charles River

Breeding Laboratories, Raleigh, NC. The rats were 9 weeks of age upon arrival and 12 weeks of age at initiation of the treatment. All rats were identified by tail tattoo and were acclimatized for three weeks. ADN-treated water or control drinking water and feed (Purina Formulab #5002, St. Louis, MO) were available *ad libitum*. Rodent drinking water was supplied via glass bottles equipped with stainless steel sipper tubes and neoprene stoppers. Bottles, stoppers, and sipper tubes were changed weekly. Animal room temperatures were targeted at 21 to 25 °C, and the light/dark cycle was set at 12-h intervals. Parental rats were single housed (except during the mating period) in clear plastic cages with hardwood-chip bedding (Bettachip®, Northeastern Products Corp., Warrensburg, NY). During the mating period the animals were housed in clear plastic cages with stainless steel wire bottoms.

General Design - Study I

Male rat reproductive toxicity was not noted in the general toxicity study, so only female rats received ADN-treated drinking water. Female rats began treatment 14 days prior to mating and continued through mating and gestation until necropsy. Mating occurred following procedures outlined in Kinkead et al., 1995. Serial sacrifice of gravid dams (10 per group) occurred on Gestation Days 10 and 20.

Body weights were measured weekly until confirmation of mating, after which they were weighed daily. Water consumption was measured except during the period of cohabitation. Dose levels were calculated from water consumption and expressed as mg ADN/kg body weight/day. At necropsy, uterine contents were examined and the number of implantations and/or resorptions was recorded. Corpora lutea were counted and the ovaries and uterus were sampled for histopathologic examination. Blood was sampled via the vena cava for clinical chemistry evaluations and whole livers were weighed at necropsy.

STUDY II

Group Assignments and ADN Treatment

Group	Number of Female Rats	ADN Treatment Period (Gestation Days)	Untreated Water Period (Gestation Days)
Preimplantation	10	1-3	4-8
Postimplantation	10	4-8	1-3
Control	10	None	1-8

Test Animals and Clinical Measurements

Thirty male and 30 female Sprague-Dawley derived outbred rats {Crl:CD®BR] known as Charles River CD rats, were purchased from Charles River Breeding Laboratories, Raleigh, NC. The animals were maintained under the conditions mentioned in Study I.

General Design - Study II

Only female rats received ADN-treated drinking water at a concentration of 2000 mg/L. No pretreatment with ADN occurred in this study. Mating occurred on the basis of one male to one female with the date of observation of a copulatory plug being designated Gestation Day 0. Mated female rats were divided into groups (N=10) and then administered ADN on a daily basis during Gestation Days 1 through 3 (the preimplantation period), or Gestation Days 4 through 8 (the postimplantation period). A group of control dams (N=9 or 10) were maintained on untreated water during Gestation Days 1 through 8. All dams were necropsied on Gestation Day 9. Body weights were measured daily, beginning on Gestation Day 0, and water consumption was measured daily during the time period the dam received treated water. At necropsy, the ovaries and uterus were weighed and blood was sampled via the vena cava for measurement of prolactin, progesterone, and estradiol.

Sera for clinical chemistry evaluations were assayed on an Ektachem 250 Analyzer (Eastman Kodak, Rochester, NY). Kit materials for the radioimmunoassays of prolactin, progesterone, and estradiol were obtained from Amersham Corp., Chicago, IL.

STATISTICS

Maternal body weights, organ weights, organ-to-body weight ratios, serum chemistry, hematology, and ADN dose calculations were analyzed for statistical significance using a one-factorial analysis of variance with Bonferroni multiple comparisons (Rosner, 1990). A one-factorial repeated measures analysis of variance with Bonferroni multiple comparisons was used for water consumption (Barcikowski, 1983). Tissue lesion severity data were analyzed using the Kruskal-Wallis analysis of variance (Rosner, 1990). Corpora lutea and fetus numbers were analyzed for statistical significance using a one-factorial analysis of variance (Barcikowski, 1983).

SECTION 3

RESULTS

STUDY I

Water consumption decreased significantly ($p<0.01$) in the high-dose rats when treatment began (Table 1) and continued to be statistically significantly less than controls throughout the gestation period. The mid-dose rats showed a decreased consumption ($p<0.05$) during the gestation period only. Water consumption of the low-dose rats compared favorably with consumption measured in the control group. Maximum water consumption occurred during the gestation period resulting in calculated mean doses of 186, 116, and 26 mg ADN/kg/day for the high-, mid-, and low-dose groups, respectively.

No mortality occurred during the study. Treatment-related differences in mean body weights were not evident during the first 10 days of gestation (Table 2). However, treatment-related differences were statistically significant beginning on Gestation Day (GD) 13 (Table 3). A similar effect was noted in absolute liver weights (Table 4). However, when the difference in body weights is accounted for, the relative weights do not show any treatment-related differences.

The only treatment-related clinical chemistry effects noted in the GD10 group of rats were an increase in blood urea nitrogen (BUN) and total bilirubin in the high-dose group only (Table 5). Clinical pathology evaluations measured at GD20 showed numerous treatment-related effects. BUN values were increased at all ADN-treatment levels (Table 6). Calcium, magnesium, total protein, and albumin were increased in the high- and mid-dose groups. Serum potassium levels were significantly decreased at the high- and mid-dose levels. No differences in total bilirubin values were noted in any of the treated groups examined at GD20.

Numbers of corpora lutea present in the ADN-treated rats at necropsy did not differ significantly from numbers noted in the control rats at either GD10 or GD20 (Table 7). The numbers of fetuses present in the uterus were greatly reduced in the female rats in the high- and mid-dose groups. Only four of the nine high-dose rats necropsied on GD10 contained fetuses. Two dams had one fetus each, one dam had six, and one dam had 11 fetuses. Four of the 10 mid-

dose dams had fetuses present in the uterus. Three had one fetus each, and the fourth rat had 10 fetuses (Appendix 1).

Two of 11 high-dose rats necropsied at GD20 had two fetuses each present in the uterus. Seven of 10 mid-dose rats had fetuses ranging in numbers from two to 17. All low-dose and control rats had fetuses at necropsy averaging 14 per animal. Overall, six of 20 high-dose, 11 of 20 mid-dose, 19 of 20 low-dose, and 20 of 20 control animals had one or more fetuses present at necropsy. Only two resorption sites were found, both in the high-dose GD20 group of rats.

At necropsy, all animals were in good general condition. Two rats (one low-dose and one control) had enlarged spleens with slight modularity, and a low-dose rat had a flat discolored spot on the liver. None of the gross observations appeared to be treatment related.

STUDY II

Mean water consumption was 29.7 mL/day and 30.4 mL/day for the preimplantation and postimplantation groups, respectively. Control animal water consumption was not measured in this study, but historically averaged 35 mL/day (Kinkead et al., 1994b). Mean doses for the two groups were 211 and 199 mg/kg/day. Treatment with ADN during Gestation Days 1 through 3 resulted in complete abolition of implantations (Table 8). ADN had no significant effect on implantations when administered during Gestation Days 4 through 8. Similarly, the implantation index (calculated as number of implantation sites/number of corpora lutea × 100) showed a significant effect in the preimplantation group but the postimplantation group was unaffected. The relative uterus weights of the preimplantation group showed a similar pattern; however, no effects on ovarian weights were noted. ADN also had no effect on the number of corpora lutea, regardless of when the rats were treated.

Serum progesterone, prolactin, and estradiol were sensitive to ADN treatment and showed a significant reduction in both ADN-treated rat groups (Table 9). All three hormones were statistically significantly ($p<0.01$) less than control values, while progesterone showed a significant ($p<0.01$) difference between the treatment groups.

SECTION 4

DISCUSSION

Rats examined at Gestation Days 10 and 20 showed a similar pattern of dose-dependent blockade of pregnancy as was noted in the initial general toxicity/reproductive screen (Kinkead et al., 1995). However, the fertility rate in the high- and mid-dose dams apparently would have been slightly higher than the initial study had normal gestation been allowed to occur. The reason for this increase is unknown. The increase in mean body weight of all dams during the first seven days was similar to what was noted in the reproductive screen. This would indicate that normal decidualization occurred; however, implantation was impaired in most instances.

In the second study, toxic effects occurred during the preimplantation period as none of the dams treated during Gestation Days 1-3 had implantation sites. Preimplantation embryolethality can be caused by death, failure of the blastocyst to form, or failure of the uterus to decidualize normally following blastocyst formation. Since normal weight gains of decidualization occurred, the latter explanation can be ruled out. It is possible that ADN effects the pituitary, thereby preventing the prolactin surges which normally occur during early pregnancy. The prolactin surges are necessary to cause the normal rise in serum progesterone secreted by the developing corpora lutea (Morishige and Rothchild, 1974). Adequate levels of progesterone are necessary for normal uterine function and the decidual growth of early pregnancy (Cummings et al., 1991). Low serum progesterone concentration prevents implantation. Serum prolactin and progesterone concentrations were also depressed in the postimplantation rat group; however, no embryolethality occurred in these animals. It is possible that implant failure could be due to other factors, i.e., oviduct motility which affects sperm, ova, and embryo transport; uterine receptivity; and ova/embryolethality. Because of the ambiguity of the hormone levels in the pre- and postimplantation rats, it is possible that the lack of implantation was due to one of these other factors. Follow-up studies are presently being conducted to determine the specific causes of the toxic effect.

Table 1. Mean Water Consumption of Female Rats Treated with Ammonium Dinitramide

	High	Medium	Low	Control
Pre-Dosing				
N	20	20	20	20
Mean	29.8	31.1	29.8	28.8
SD	3.7	5.1	3.6	6.3
SEM	0.8	1.1	0.8	1.4
Median	29.5	30.9	30.7	27.0
Pre-Mating				
N	20	20	20	20
Mean	22.6[a]	27.8	29.1	28.2
SD	4.1	3.2	4.0	5.3
SEM	0.9	0.7	0.9	1.2
Median	21.7	27.4	29.9	27.0
Gestation				
N	18	20	20	20
Mean	28.3[a]	35.8[b]	41.0	41.3
SD	5.2	2.6	5.3	8.3
SEM	1.2	0.6	1.2	1.9
Median	28.2	35.6	41.7	37.9

[a]Significantly different from control at $p<0.01$.
[b]Significantly different from control at $p<0.05$.

Table 2. Mean Body Weights[a] of Female Rats Treated with Ammonium Dinitramide Sacrificed on Gestation Day 10

Day of Study	Control	Low	Medium	High[b]
PM0	263.1 ± 4.7	264.7 ± 5.4	269.3 ± 5.5	270.6 ± 4.8
PM7	273.3 ± 5.8	279.1 ± 7.0	280.1 ± 7.5	282.9 ± 5.0
PM14	285.1 ± 7.6	289.4 ± 8.7	290.4 ± 7.0	290.8 ± 6.6
G0	283.1 ± 7.5	289.9 ± 8.4	283.9 ± 8.6	288.1 ± 6.4
G1	290.5 ± 7.3	295.0 ± 9.1	290.5 ± 8.2	291.8 ± 6.4
G2	299.8 ± 7.5	303.2 ± 9.2	297.8 ± 8.4	301.2 ± 6.0
G3	305.8 ± 7.7	308.8 ± 9.7	302.7 ± 8.8	306.1 ± 6.0
G4	310.4 ± 7.5	314.0 ± 10.0	305.8 ± 8.6	310.2 ± 6.2
G5	316.2 ± 7.6	317.6 ± 9.7	309.2 ± 9.2	314.0 ± 6.5
G6	320.1 ± 7.1	323.7 ± 9.5	311.1 ± 9.1	319.1 ± 6.4
G7	322.8 ± 7.3	326.1 ± 9.6	314.6 ± 9.4	322.1 ± 6.5
G8	327.1 ± 7.5	330.6 ±10.0	317.0 ± 10.0	323.4 ± 6.1
G9	332.4 ± 7.2	335.7 ± 9.1	318.5 ± 10.3	328.1 ± 7.0
G10	336.3 ± 7.8	334.5 ± 11.1	320.5 ± 12.9	334.6 ± 7.4

[a]Mean ± SEM, N = 10.
[b]N = 9.
PM = Premating
G = Gestation

Table 3. Mean Body Weights[a] of Female Rats Treated with Ammonium Dinitramide Sacrificed on Gestation Day 20

Day of Study	Control	Low	Medium	High[b]
PM0	271.5 ± 5.0	268.6 ± 4.6	264.1 ± 4.1	265.4 ± 6.1
PM7	279.7 ± 4.8	279.7 ± 5.0	274.1 ± 5.7	268.8 ± 7.4
PM14	296.3 ± 5.5	289.1 ± 4.2	285.4 ± 6.4	276.5 ± 8.1
G0	296.3 ± 5.0	291.5 ± 4.7	283.6 ± 7.2	275.1 ± 8.9
G1	299.2 ± 4.4	297.0 ± 4.0	290.3 ± 7.0	279.4 ± 9.3
G2	309.1 ± 4.6	304.2 ± 4.4	298.1 ± 7.0	288.8 ± 9.8
G3	315.2 ± 3.8	309.1 ± 4.2	302.8 ± 7.0	294.2 ± 9.7
G4	318.9 ± 3.0	314.1 ± 4.0	310.1 ± 7.2	298.8 ± 10.1
G5	322.9 ± 3.6	320.6 ± 4.5	313.9 ± 7.4	303.7 ± 10.7
G6	325.2 ± 3.8	325.7 ± 4.7	317.3 ± 7.6	304.1 ± 10.7
G7	329.1 ± 4.2	327.9 ± 5.4	320.1 ± 7.5	308.0 ± 10.8
G8	333.8 ± 4.2	331.0 ± 4.5	322.7 ± 7.4	311.8 ± 11.3
G9	337.9 ± 3.6	337.0 ± 5.2	326.7 ± 7.7	314.9 ± 11.8
G10	342.0 ± 4.2	343.7 ± 6.0	330.7 ± 7.2	317.6 ± 11.4
G11	349.7 ± 3.6	350.1 ± 6.0	334.4 ± 7.3	321.5 ± 11.5
G12	352.6 ± 3.4	355.0 ± 5.8	339.2 ± 6.9	323.4 ± 12.3
G13	356.9 ± 3.2	359.5 ± 6.7	342.2 ± 6.9	324.1 ± 13.1[c]
G14	360.4 ± 3.7	364.7 ± 6.1	343.0 ± 7.0	324.4 ± 13.5[c]
G15	368.6 ± 4.3	373.1 ± 7.2	342.4 ± 6.4	322.0 ± 14.0[d]
G16	379.0 ± 4.3	385.1 ± 7.8	345.9 ± 6.5	320.3 ± 14.9[d]
G17	393.1 ± 5.0	396.7 ± 9.1	350.7 ± 6.6[c]	321.2 ± 14.1[d]
G18	409.3 ± 5.1	414.6 ± 9.5	356.3 ± 7.5[d]	323.2 ± 14.4[d]
G19	424.7 ± 5.0	428.9 ± 9.8	363.1 ± 8.2[d]	322.8 ± 14.5[d]
G20	439.8 ± 5.8	447.3 ± 11.3	372.3 ± 10.0[d]	322.8 ± 15.9[d]

[a]Mean ± SEM, N = 10.
[b]N = 11.
[c]Different from control at p<0.05.
[d]Different from control at p<0.01.
PM = Premating
G = Gestation

Table 4. Absolute (g)[a] and Liver-to-Body Weight Ratios[b] of Female Rats Treated with Ammonium Dinitramide through Gestation Days 10 and 20

	Control	Low	Medium	High
G10				
Liver	14.0 ± 0.5	13.6 ± 0.3	13.1 ± 0.5	13.3 ± 0.5[c]
Ratio	4.2 ± 0.5	4.0 ± 0.1	4.2 ± 0.1	4.0 ± 0.1[c]
Body weight	339.0 ± 8.4	341.6 ± 8.0	324.2 ± 11.2	330.9 ± 7.0[c]
G20				
Liver	16.0 ± 0.5	18.6 ± 0.5[e]	14.6 ± 0.6[e]	12.2 ± 0.6[de]
Ratio	3.7 ± 0.1	4.2 ± 0.1[f]	4.0 ± 0.1	3.8 ± 0.1[d]
Body weight	432.1 ± 12.7	444.1 ± 11.1	367.5 ± 10.1[e]	318.3 ± 11.6[de]

[a]Mean ± SEM, N = 10.
[b]Liver weight/body weight × 100.
[c]N = 9.
[d]N = 11.
[e]Significantly different from control at $p<0.01$.
[f]Significantly different from control at $p<0.05$.

Table 5. Mean Values[a] of Clinical Chemistry Parameters for Female Rats Treated with Ammonium Dinitramide through Gestation Day 10

	Control	Low	Medium	High[b]
Glucose (mg/dL)	201.8 ± 5.3	214.9 ± 8.1	200.5 ± 12.4	196.3 ± 7.4
BUN (mg/dL)	17.2 ± 0.8	18.0 ± 0.8	19.1 ± 0.8	21.7 ± 0.8[c]
Creatinine (mg/dL)	0.6 ±<0.1	0.7 ±<0.1	0.7 ±<0.1	0.9 ± 0.2
Sodium (mmol/L)	143.7 ± 0.4	144.6 ± 0.4	145.1 ± 0.6	147.3 ± 1.5
Potassium (mmol/L)	5.2 ± 0.2	5.1 ± 0.2	4.1 ± 0.2[c]	5.2 ± 0.8
Chloride (mmol/L)	97.4 ± 0.6	98.0 ± 0.4	97.0 ± 0.8	101.8 ± 3.8
CO_2 (mmol/L)	38.2 ± 0.4	37.6 ± 0.6	37.8 ± 0.7	38.2 ± 0.6
Calcium (mg/dL)	11.9 ± 0.1	11.8 ± 0.1	12.0 ± 0.1	11.6 ± 0.3
Magnesium (mg/dL)	3.6 ± 0.2	3.3 ± 0.1	3.5 ± 0.2	4.0 ± 0.3
Phosphorus (mg/dL)	9.5 ± 0.3	9.7 ± 0.3	9.5 ± 0.4	8.8 ± 0.3
Cholesterol (mg/dL)	62.3 ± 2.6	58.6 ± 4.0	65.6 ± 3.1	62.0 ± 3.5
Triglycerides (mg/dL)	118.5 ± 6.9	112.6 ± 10.1	136.5 ± 11.7	154.9 ± 24.6
Total Protein (g/dL)	6.7 ± 0.1	6.8 ±<0.1	7.0 ± 0.1	6.9 ± 0.1
Albumin (g/dL)	3.9 ± 0.1	3.9 ± 0.1	4.1 ± 0.1	4.2 ± 0.1
AST (IU/L)	170.6 ± 19.3	185.1 ± 20.7	163.5 ± 15.6	171.4 ± 22.9
ALT (IU/L)	62.9 ± 4.9	65.5 ± 6.3	52.5 ± 3.2	45.2 ± 6.1
LDH (IU/L)	406.3 ± 57.6	400.5 ± 48.2	398.4 ± 40.0	498.8 ± 32.4
Creatine Kinase (IU/L)	107.3 ± 11.2	86.5 ± 9.1	98.8 ± 9.3	104.2 ± 8.5
Alkaline phosphatase (IU/L)	259.6 ± 21.4	244.7 ± 19.3	275.8 ± 24.4	240.8 ± 28.6
Total Bilirubin (mg/dL)	0.2 ±<0.1	0.3 ±<0.1	0.3 ±<0.1	0.4 ±<0.1[c]
Uric Acid (mg/dL)	2.4 ± 0.2	2.4 ± 0.2	2.2 ± 0.2	2.1 ± 0.2
A/G Ratio	1.3 ±<0.1	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1

[a]Mean ± SEM, N = 10.
[b]N = 9.
[c]Significantly different from control at $p<0.01$.

Table 6. Mean Values[a] of Clinical Chemistry Parameters for Female Rats Treated with Ammonium Dinitramide through Gestation Day 20

	Control	Low	Medium	High[b]
Glucose (mg/dL)	111.3 ± 7.2	109.0 ± 5.0	145.3 ± 17.9	205.1 ± 15.8[c]
BUN (mg/dL)	14.3 ± 0.6	18.1 ± 0.9[d]	19.3 ± 1.0[c]	20.0 ± 1.6[c]
Creatinine (mg/dL)	0.8 ± 0.1	0.7 ±<0.1	0.8 ±<0.1	0.7 ± 0.1
Sodium (mmol/L)	139.4 ± 1.1	137.8 ± 1.0	141.2 ± 1.0	142.8 ± 1.3
Potassium (mmol/L)	5.7 ± 0.2	5.3 ± 0.1	4.4 ± 0.2[c]	4.6 ± 0.2[c]
Chloride (mmol/L)	96.3 ± 1.0	94.7 ± 1.2	94.2 ± 1.0	95.7 ± 1.2
CO_2 (mmol/L)	35.8 ± 1.0	36.4 ± 1.0	37.8 ± 1.0	36.7 ± 0.9
Calcium (mg/dL)	10.4 ± 0.1	10.8 ± 0.1	11.3 ± 0.2[c]	11.9 ± 0.1[c]
Magnesium (mg/dL)	2.6 ± 0.1	2.8 ± 0.1	3.1 ± 0.1[c]	3.2 ± 0.1[c]
Phosphorus (mg/dL)	5.4 ± 0.3	6.4 ± 0.4	7.0 ± 0.5	7.5 ± 0.3
Cholesterol (mg/dL)	78.1 ± 4.6	79.4 ± 4.8	69.0 ± 4.5	58.3 ± 4.7[c]
Triglycerides (mg/dL)	516.8 ± 91.5	528.3 ± 68.9	371.2 ± 69.9	176.8 ± 35.8[d]
Total Protein (g/dL)	6.3 ± 0.2	6.2 ± 0.1	7.2 ± 0.2[d]	7.0 ± 0.1[d]
Albumin (g/dL)	3.3 ± 0.1	3.3 ± 0.1	4.1 ± 0.2[c]	4.0 ± 0.1[c]
AST (IU/L)	290.2 ± 33.0	218.5 ± 35.9	266.4 ± 38.5	204.8 ± 39.6
ALT (IU/L)	51.9 ± 2.2	59.4 ± 3.5	64.2 ± 4.3	66.2 ± 14.3
LDH (IU/L)	467.0 ± 84.5	429.2 ± 29.7	471.5 ± 22.4	454.9 ± 77.6
Creatine Kinase (IU/L)	140.0 ± 10.9	132.8 ± 29.0	132.7 ± 10.8	124.6 ± 31.9
Alkaline phosphatase (IU/L)	147.0 ± 19.0	196.4 ± 21.9	202.0 ± 27.4	175.3 ± 17.3
Total Bilirubin (mg/dL)	0.3 ±<0.1	0.3 ±<0.1	0.3 ±<0.1	0.3 ±<0.1
Uric Acid (mg/dL)	1.8 ± 0.1	1.9 ± 0.2	2.0 ± 0.2	2.3 ± 0.2
A/G Ratio	1.1 ±<0.1	1.2 ±<0.1	1.3 ± 0.1[d]	1.3 ± 0.1[d]

[a]Mean ± SEM, N = 10.
[b]N = 9.
[c]Significantly different from control at $p<0.01$.
[d]Significantly different from control at $p<0.05$.

Table 7. Corpora Lutea and Fetus Counts[a] from Rats Treated with Ammonium Dinitramide Before and During Gestation

Rats were maintained through either 10 or 20 days gestation

Treatment	Gestation Day 10		Gestation Day 20	
	Corpora lutea	Fetuses	Corpora lutea	Fetuses
High	17.2 ± 0.9	2.1 ± 1.3[b]	21.3 ± 2.2	0.4 ± 0.2[b]
Medium	16.0 ± 1.0	1.3 ± 1.0[b]	19.6 ± 1.8	4.6 ± 1.8[b]
Low	16.1 ± 1.0	15.1 ± 1.8	19.5 ± 1.0	14.4 ± 0.8
Control	17.2 ± 0.7	17.1 ± 0.7	17.7 ± 0.8	14.1 ± 0.8

[a]Mean ± SEM.
[b]Significantly different from control at $p<0.01$.

Table 8. Effects of ADN Treatment During Early Pregnancy[a]

Treatment Period	N	Ovarian Weight (mg)	Uterus Weight (g)	Number of Corpora Lutea	Number of Implantation Sites	Implantation Index
1 - 3	10	109.1 ± 17	0.38 ± 0.15[b]	19.0 ± 1.6	0.0[b]	0.0[b]
4 - 8	10	107.0 ± 19	1.79 ± 0.13[c]	17.6 ± 0.8	16.4 ± 0.5	93.2
None	9	118.4 ± 28	1.44 ± 0.38	17.3 ± 1.0	15.2 ± 1.5	87.9

[a]ADN was administered to groups of rats on either Gestation Days 1-3 or 4-8, or not at all (control). Parameters were assessed following euthanasia on Gestation Day 9. Data are presented as Mean ± SEM except for Implantation Index.
[B]Significantly different from control at $p<0.01$.
[c]Significantly different from control at $p<0.05$.

Table 9. Effects of ADN Treatment on Serum Progesterone, Prolactin, and Estradiol Levels

Treatment Period	N	Estradiol (pg/mL)	Progesterone (ng/mL)	Prolactin (ng/mL)
1 - 3	10	4.43 ± 0.16[a]	111.11 ± 2.40[ab]	79.99 ± 2.04[a]
4 - 8	10	3.46 ± 0.06[a]	128.80 ± 1.49[a]	75.34 ± 1.70[a]
None	9	5.27 ± 0.11	140.65 ± 1.19	92.82 ± 2.41

[a]Significantly different from control at $p<0.01$.
[b]Significantly different from postimplantation group at $p<0.01$.

SECTION 5

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APPENDIX 1

Dose Group (mg/kg/day)	Animal Number	Corpora lutea	Fetuses
High	307	16	0
160 (10 days)	331	22	11
	317	15	6
	338	20	0
	305	16	0
	311	19	1
	356	16	0
	372	18	0
	363	13	1
Medium	306	15	0
100 (10 days)	325	20	0
	339	16	0
	328	21	0
	335	12	0
	346	19	1
	340	11	0
	364	14	10
	358	16	1
	362	16	1
Low	316	14	0
30.0 (10 days)	309	17	16
	327	9	18
	301	15	15
	321	15	15
	330	18	17
	350	17	17
	355	19	18
	348	16	15
	373	21	20
Control	323	20	17
0.0 (10 days)	304	16	16
	314	15	15
	332	19	21
	310	19	17
	360	16	19
	365	18	18
	374	14	13
	380	15	18
	379	20	17

APPENDIX 1 cont'd

Dose Group (mg/kg/day)	Animal Number	Corpora lutea	Fetuses
High	324	16	0
160 (20 days)	320	13	2
	354	28	0
	359	19	0
	366	21	0
	367	15	2
	371	30	0
	377	27	0
	361	34	0
	302	16	0
	349	15	0
Medium	313	19	3
100 (20 days)	326	14	3
	318	16	12
	333	27	0
	336	31	0
	352	18	3
	351	22	0
	353	14	2
	378	19	17
	344	16	6
Low	308	16	15
30.0 (20 days)	319	25	16
	329	17	15
	322	18	16
	347	19	16
	343	16	16
	337	23	12
	370	23	11
	376	17	17
	368	21	10
Control	334	17	11
0.0 (20 days)	312	14	13
	315	21	11
	341	23	16
	357	17	15
	342	19	18
	345	17	17
	369	17	16
	375	16	12